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POSTSYNAPTIC DENSITY: A KEY CONVERGENT SITE FOR SCHIZOPHRENIA SUSCEPTIBILITY FACTORS AND POSSIBLE TARGET FOR DRUG DEVELOPMENT

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Summary

Many studies have supported roles for both genetic and environmental factors in the etiology of schizophrenia. A major hypothesis at present is that schizophrenia is a polygenic disorder where alterations in a set of genes lead to impaired neurodevelopment, which in turn results in altered neurotransmission. Several neurotransmitters, including glutamate, dopamine, serotonin and gamma-amino butyric acid (GABA), have been implicated in schizophrenia, and, as such, there is a growing interest in trying to elucidate the mechanisms whereby alterations in the function of schizophrenia susceptibility gene products can lead to disturbance in signaling at synapses. In this article, we will summarize what is known about schizophrenia susceptibility factors that reside at postsynaptic density (PSD), a unique postsynaptic site where signals from neurotransmitters converge. PSD may be a promising target for novel classes of drugs to treat schizophrenia.

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Introduction

Schizophrenia is a complex neuropsychiatric condition affecting about 1% of the population worldwide. Its etiology remains unclear, although genetic and epidemiological studies suggest that the interplay between genetic and environmental factors during the early stages of neurodevelopment could contribute to its pathogenesis (1, 2). Recently, systematic linkage and association studies have provided a list of susceptibility genes that include *neuregulin (NRG)-1, dysbindin, disrupted-in-schizophrenia 1 (DISC1), neuronal nitric oxide synthase (nNOS)* and *carboxyl-terminal PDZ ligand of neuronal nitric oxide synthase (CAPON)* (3–5) (Table I).

Brain imaging studies of patients and neuropathological approaches have revealed that schizophrenia patients present with enlarged lateral ventricles, decreased brain volumes and altered synaptic morphology including decreased dendritic spines and arborizations (3, 6–12). These neuroanatomical disturbances have led some to postulate that impaired neurodevelopment may lead to altered neurotransmission in schizophrenia. Interestingly, the majority of genetic susceptibility factors for schizophrenia localize to postsynaptic density (PSD), a unique postsynaptic site, where they may converge and modulate signals downstream of various neurotransmitter receptors (Fig. 1). Of all the neurotransmitters thought to contribute to the pathophysiology of schizophrenia, glutamate has received the most attention. This is because numerous studies have demonstrated that hypoglutamatergic signaling is associated with psychosis. Some studies have found that phencyclidine, a *N*-methyl-*D*-aspartate (NMDA) receptor antagonist, induces schizophrenia-like behaviors in humans (13). In addition, studies in mice, rats and monkeys treated with phencyclidine have shown that these animals displayed behavioral and/or neuroanatomical deficits that resemble those seen in schizophrenia patients (14–16). Thus, PSD linked to NMDA receptors may be a site of convergence for the disease pathways, and, as such, it may be an important target for new drugs to treat schizophrenia. Once we would have identified signaling pathways altered in schizophrenia and their phenotypic outcomes, we can design agents to alleviate or treat these phenotypic insults.

In this review, we will focus on genetic susceptibility factors for schizophrenia that may play a role at PSD associated with NMDA receptors, and discuss potential therapeutic interventions at this site. Our discussion will be centered on the function of these gene products as well as genetic studies linking alterations in these genes to abnormal phenotypes in humans.

Neuregulin-1 and ErbB4

Function

NRG-1 belongs to the family of proteins containing an epidermal growth factor–like motif that is known to activate ErbB1–4 receptors tyrosine ki-

Table I: Susceptibility genes for schizophrenia.

Gene	Chromosomal locus	Function
CAPON	1q22	Regulates the coupling of nNOS to the NMDA receptor via PSD-95
DISC1	1q42	Neurites outgrowth
		Neuronal migration
		Axonal elongation
		Modulates cyclic AMP pathway
Dysbindin	6p22.3	Modulates dopamine release
		Stimulates glutamate release
		Upregulates presynaptic proteins
		Neurotrophic effect through Akt signaling pathway
NRG-1	8p22	Neuronal migration and differentiation
		Modulates $GABA_A$ receptor expression
		Recruits CHRNA7 to synapses
		Regulates expression and plasticity of NMDA receptor
nNOS	12q24	Second messenger of NMDA receptor
		Modulates dopamine and serotonin neurotransmission

NMDA = *N*-methyl-*D*-aspartate; Akt = protein kinase B; GABA = gamma-amino butyric acid.

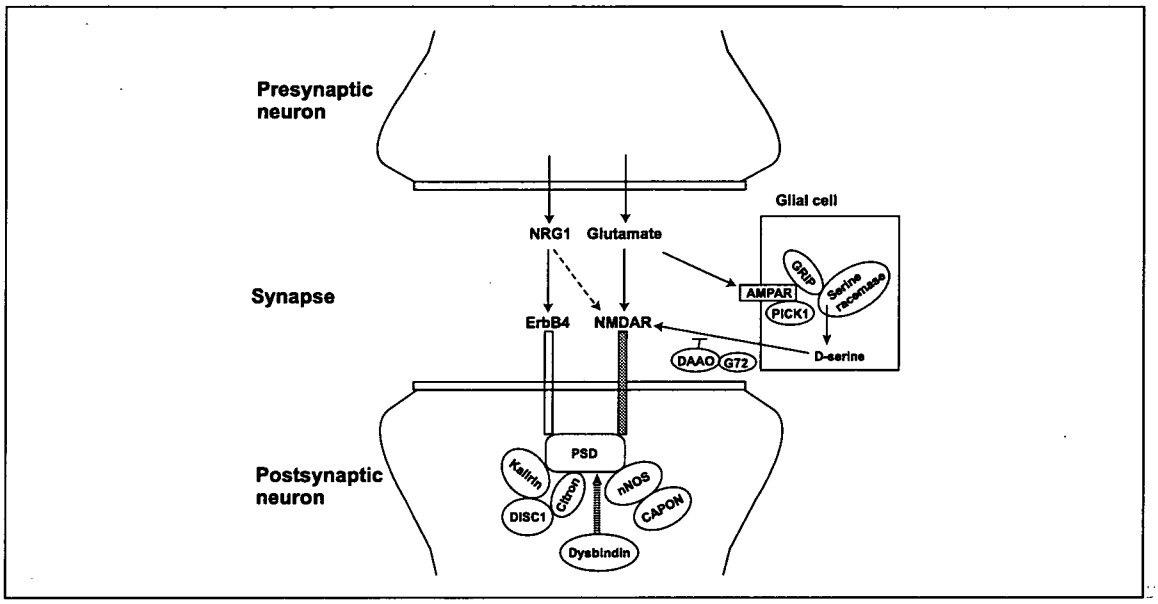

Fig. 1. Schematic representation of schizophrenia gene products localizing at postsynaptic density (PSD). Neuregulin (NRG)-1, dysbindin, disrupted-in-schizophrenia 1 (DISC1), neuronal nitric oxide synthase (nNOS) and carboxyl-terminal PDZ ligand of neuronal nitric oxide synthase (CAPON) are schizophrenia susceptibility gene products that reside at PSD associated with *N*-methyl-*D*-aspartate receptor (NMDAR). GRIP = glutamate receptor-interacting protein; AMPAR = alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptor; PICK1 = protein interacting with C kinase 1.

nase (17, 18). NRG-1 has nine known splicing variants that are all expressed in human brain (19). The NRG-1 receptor, ErbB4, has been well characterized. Of most importance, ErbB4 occurs in PSD where it interacts with PSD95, an anchoring protein of the NMDA receptor (20, 21). ErbB4 has several isoforms that are expressed in the brain (22). Splicing variants containing exon 16 encode for ErbB4 isoforms carrying a metalloprotease-sensitive extracellular domain referred to as JM-a. Splicing variants containing exon 26 encode for a cytoplasmic domain containing a phosphotidylinositol-3 kinase (PI3K) binding site referred to as CYT-1.

NRG-1 regulates the expression of three receptors that have been implicated in the pathophysiology of schizophrenia: NMDA, gamma-amino butyric acid (GABA) and nicotinic acetylcholine receptors (23–26). Studies using cerebellar slice cultures have shown that a NRG-beta isoform stimulates the expression of NR2C, an NMDA receptor subunit (27). As discussed below, studies with human tissue have demonstrated a novel function of NRG-1–ErbB4 interaction.

Human studies

Ten independent groups found a genetic association between the *NRG-1* gene and schizophrenia (28–37). A recent study reported for the first time that an NRG-1 variant in individuals at high risk for schizophrenia is associated with impaired activation of frontal and temporal lobes, lower intelligence quotient and increased susceptibility for psychotic symptoms (38).

A study using autopsied human brains reported that type I NRG-1 mRNA is increased in the dorsolateral prefrontal cortex of schizophrenia patients (39). Similar results were obtained in the hippocampus of a much larger and separate sample of schizophrenia patients (40).

Studies using postmortem brains from schizophrenia patients also reported a decrease in the number of oligodendrocytes (41–45). Consistent with these reports, microarray studies have also reported a general reduction in the expression of oligodendrocyte- and myelinization-associated genes in schizophrenia patients (46–48). Since NRG-1 plays an important role in oligodendrocytes development, NRG-1 dysfunction could lead to poor myelination and thus impaired synaptic for-

mation due to improper oligodendrocyte development.

Even though numerous genetic studies in schizophrenia patients have found that the *NRG-1* gene is associated with schizophrenia, we still do not know how alteration in NRG-1 function can lead to the disorder. A study by Hahn *et al.* (49) using postmortem brains from schizophrenia patients suggests that upregulation of NRG-1–ErbB4 signaling suppresses NMDA receptor activation more in schizophrenia patients than in healthy controls. Other studies supporting an association between enhanced NRG-1–ErbB4 signaling and schizophrenia include a report showing a genetic interaction between the Icelandic *NRG-1* haplotype and ErbB4 (37). Another study reported that two ErbB4 isoforms, JM-a and CYT-1, were overexpressed in the dorsolateral prefrontal cortex of schizophrenia patients from their Ashkenazi cohort (50). CYT-1 is a known activator of the PI3K–protein kinase B (Akt) pathway that has been implicated in some cases of schizophrenia.

DISC1

Function

The *DISC1* gene was originally identified at the breakpoint on chromosome 1 resulting from a balanced chromosomal translocation between chromosomes 1 and 11 in a large Scottish family with hereditary psychiatric disorders, including schizophrenia (51, 52). DISC1 is multifunctional and has several subcellular distributions (53). In proliferating cells and developing neurons, a pool of DISC1 forms a complex with dynein, nuclear distribution element-like, and lissencephaly 1 protein at the centrosome (54). Disruption of this complex following suppression of DISC1 expression by RNA interference leads to improper neuronal migration and abnormal dendritic arborizations in the cerebral cortex *in vivo* (54). DISC1 may also function to modulate the cyclic AMP (cAMP) pathway by binding to phosphodiesterase 4B, which itself was found to be disrupted by a balanced chromosomal translocation in two patients with schizophrenia (55). The cAMP pathway is known to modulate learning, memory and mood, and, as such, the interaction between DISC1 and PDE4B suggests that DISC1 may play a role in synaptic plasticity (56–58).

In adult brains, DISC1 turns out to be enriched in PSD and the nucleus. A study demonstrated by immunoelectron microscopy that DISC1 was present in about 8% of axon terminals in the frontal and parietal cortex of healthy subjects (59) (Fig. 2). Furthermore, yeast two-hybrid studies have shown that DISC1 interacts with numerous PSD proteins, including Citron (60, 61).

Human studies

Association of DISC1 with schizophrenia in general has also been supported by various genetic studies (53, 62). For example, a *DISC1* haplotype was found to be undertransmitted in a North American Caucasian population with schizoaffective disorder (63). The same study also reported that haplotype blocks located within exons 1 and 9 are associated with various neuropsychiatric disorders, including schizophrenia, and that the Phe607 allele is overtransmitted in patients with schizoaffective disorders. Another study showed that the Ser704 DISC1 allele is overtransmitted among schizophrenia patients, and that in healthy individuals this allele was associated with defective hippocampal formation and function (64). In addition, several studies have shown that the Ser704Cys DISC1 polymorphism is associated with impaired cognitive function (64–66).

Another study reported that an asymptomatic carrier of the Scottish mutation presented with a deficit in P300 event-related potential, which indicates impairment in higher processes such as memory and attention (67).

Deficits in spatial and working memory function as well as reduced gray matter volume in the dor-

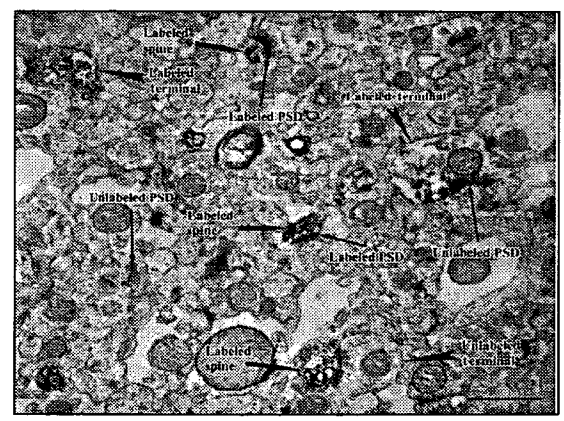

Fig. 2. Electron micrograph showing DISC1 immunoreactivity in various postsynaptic densities (PSD). Reproduced from ref. 59 with permission from John Wiley & Sons, Inc. © 2006.

solateral prefrontal cortex were found in individuals who carry the Finnish *DISC1* risk haplotype, HEP1 (68). Another *DISC1* haplotype, HEP3, has been associated with impaired visual memory and attention (69).

In postmortem brains, we reported that disturbance in the nuclear pool of DISC1 is observed in schizophrenia patients as well as in alcohol/substance abuse patients (70).

Dysbindin

Function

Dysbindin was originally found as a binding partner of alpha- and beta-dystrobrevin, which are causative genes of Duchenne muscular dystrophy (71). Dystrobrevins exist in the dystrophin-associated protein complex that is important for proper muscle function (72). Lack of dystrophin leads to alterations in neuronal membranes that in turn can cause cognitive deficits in affected individuals (73).

Little is known about the functions of dysbindin in neurons. It has been shown that suppression of dysbindin expression in PC12 cells resulted in an increase of dopamine release (74). Another study showed that dysbindin might influence exocytotic glutamate release via upregulation of the presynaptic machinery molecules (75). The same study also reported that dysbindin promotes neuronal viability through PI3K–Akt signaling. A recent study demonstrated that dysbindin interacts with snapin, and the two proteins colocalize in presynaptic sites rich in synaptic vesicle membrane as well as in PSD (76). More studies are needed to elucidate a possible role for dysbindin at PSD.

Human studies

Numerous studies have reported an association between genetic variants of dysbindin and schizophrenia in several populations (75, 77–83).

Some studies have shown that dysbindin single-nucleotide polymorphisms (SNPs) are associated with impairments in general cognitive ability, frontal brain function, as well as more severe negative symptoms in healthy subjects and patients with schizophrenia (84–86). For instance, a study conducted on 213 patients with schizophrenia or schizoaffective disorder found that dysbindin genetic variants that have been associated with schizophrenia can affect intelligence (87). Another study found an association between a dysbindin risk haplotype and spatial working memory (88).

Studies with human autopsied brains have revealed alterations in levels of dysbindin mRNA and protein. One study reported that dysbindin expression is reduced in the hippocampus of schizophrenia patients (89). This reduction was more prominent in the inner molecular layer of the dentate gyrus and correlated with an increase in vesicular glutamate transporter-1. These results suggest that altered glutamatergic signaling may lead to improper hippocampal formation. Another study found decreased dysbindin mRNA levels in the prefrontal cortex and midbrain of schizophrenia patients (90).

nNOS and CAPON

Function

The neuronal form of nitric oxide synthase, also known as neuronal nicotinamide-adenine dinucleotide phosphate-diaphorase (NADPH-d), is the major source of nitric oxide in the central nervous system (91, 92). Nitric oxide is known to function as a second messenger downstream of the NMDA receptor (93). Signal downstream of the NMDA receptor is mediated in part by nNOS, since calcium entering through NMDA receptor channels binds to the calmodulin–nNOS protein complex. CAPON, a new risk factor for schizophrenia, is an adapter protein mediating the coupling of nNOS to the NMDA receptor via PSD95 protein (94).

Human studies

nNOS gene polymorphism has been associated with schizophrenia (95–97). A study showed that the alternative NOS1 exons 1c and 1f are associated with psychosis and altered prefrontal cortex function (96). Another study conducted on 215 Japanese patients with schizophrenia and 182 healthy subjects found an association between an SNP located in exon 29 of the *nNOS* gene and schizophrenia (97). However, another study reported that they did not find an association between a CA dinucleotide repeat polymorphism in the 3'-UTR exon 29 of the nNOS gene and schizophrenia in a Chinese cohort (98).

A study reported various alterations in the morphology as well as the number of NOS-positive neurons in the striatum of schizophrenia patients as measured by immunohistochemistry (99). Another study reported that schizophrenia patients have a reduced number of NADPH-d–positive neurons in the hippocampal formation and neocortex of the lateral temporal lobe (100). This study al-

so found that in schizophrenia patients, the number of NADPH-d–positive neurons was increased in the white matter of the lateral temporal lobe as well as in parahippocampal white matter. There has also been a report of altered distribution of NADPH-d–positive neurons in the frontal lobe of schizophrenia patients (101).

One study reported that patients with schizophrenia and those with bipolar disorder have increased expression of CAPON in the dorsolateral prefrontal cortex (102). According to the authors of this study, one of the consequences associated with increased expression of CAPON is NMDA receptor hypofunction due to disturbance of the interaction between nNOS and NMDA receptor.

Conclusions

NRG-1, DISC1, dysbindin, nNOS and CAPON are examples of key schizophrenia susceptibility factors that could modulate glutamatergic signaling at PSD. Alteration in these factors may mediate the disease-associated disturbance of NMDA–glutamatergic signaling. Thus, agents that can modulate signaling involving these factors at PSD to enhance overall glutamate signaling could potentially be efficacious drugs to treat schizophrenia. Findings from human genetic studies have allowed scientists to make genetically engineered mice displaying schizophrenia-like symptoms, which can be used to elucidate the mechanisms whereby schizophrenia susceptibility genes contribute to its pathophysiology as well as to screen novel classes of drugs for the treatment of schizophrenia.

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The Dysbindin Gene (*DTNBP1*) Is Associated with Methamphetamine Psychosis

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Background: The dysbindin (*DTNBP1* [dystrobrevin-binding protein 1]) gene has repeatedly been shown to be associated with schizophrenia across diverse populations. One study also showed that risk haplotypes were shared with a bipolar disorder subgroup with psychotic episodes, but not with all cases. *DTNBP1* may confer susceptibility to psychotic symptoms in various psychiatric disorders besides schizophrenia.

Methods: Methamphetamine psychosis, the psychotic symptoms of which are close to those observed in schizophrenia, was investigated through a case (n = 197)–control (n = 243) association analyses of *DTNBP1*.

Results: *DTNBP1* showed significant associations with methamphetamine psychosis at polymorphisms of P1635 (rs3213207, $p = .00003$) and SNPA (rs2619538, $p = .049$) and the three-locus haplotype of P1655 (rs2619539)-P1635-SNPA (permutation $p = .0005$). The C-A-A haplotype, which was identical to the protective haplotype previously reported for schizophrenia and psychotic bipolar disorders, was a protective factor ($p = .0013$, odds ratio [OR] = .62, 95% confidence interval [CI] .51–.77) for methamphetamine psychosis. The C-G-T haplotype was a risk for methamphetamine psychosis ($p = .0012$, OR = 14.9, 95% CI 3.5–64.2).

Conclusions: Our genetic evidence suggests that *DTNBP1* is involved in psychotic liability not only for schizophrenia but also for other psychotic disorders, including substance-induced psychosis.

Key Words: Akt1, *DTNBP1*, dysbindin, methamphetamine psychosis, substance dependence

A genetic variation of the dystrobrevin-binding protein 1 (*DTNBP1*) gene has recently been shown to be associated with schizophrenia in several independent studies. Straub *et al.* (1) revealed original evidence for a positive genetic association between schizophrenia and variants in a gene on 6p22.3, dysbindin (*DTNBP1*), which is located within one of several promising loci revealed by a genomewide linkage scan. Many replication studies showed consistent findings in different populations, for example, German (2), Irish (3), Chinese (4), Swedish/German/Polish (5), UK/Irish (5), Bulgarian (6), American (7), Scottish/Chinese (8), and Japanese (9), although the significantly associated alleles and haplotypes were not always consistent among populations. Two postmortem studies also revealed that dysbindin protein or its mRNA level was reduced in the dorsolateral prefrontal cortex and in presynaptic glutamatergic terminals of the hippocampus of schizophrenia patients (10,11). These findings suggest that the dysbindin is involved in the pathogenesis of schizophrenia.

Recently, Raybould *et al.* (12) examined three loci of the *DTNBP1* gene in a large sample of patients with bipolar disorder, another endogenous psychosis, in UK Caucasians, and found that the *DTNBP1* gene was not associated with all cases of bipolar disorder but was associated with a subgroup of bipolar disorder characterized by the complication of psychotic features during episodes. The risk and protective haplotype were identical to those found in their previous schizophrenia study (13). Therefore, they speculated that the *DTNBP1* genetic variation influences susceptibility to schizophrenia and bipolar psychosis across the Kraepelinian dichotomy.

Abuse of large amounts of methamphetamine for long periods easily produces psychotic symptoms, such as delusions of reference, persecution, and poisoning, as well as auditory and visual hallucinations (14–16). Further consumption of methamphetamine may result in severe psychosis, liability to relapse with reconsumption of methamphetamine or psychological stress, and a gradually worsening prognosis. Clinical similarities between methamphetamine psychosis and schizophrenia in a cross-section of clinical features have been noted; these include auditory hallucination and delusion, the longitudinal process of progressive exacerbation with acute relapses, relatively good response to neuroleptics, and enduring vulnerability to relapse to stressors, especially in the paranoid type of schizophrenia. Indeed, methamphetamine psychosis has long been considered a pharmacologic model of schizophrenia (17,18), and shared molecular mechanisms could be involved in these psychotic disorders. Based on this rationale, it is possible that the *DTNBP1*

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gene may influence susceptibility to substance-induced psychoses in the same manner that influence susceptibility to schizophrenia and bipolar psychosis disorders. To examine this hypothesis, we investigated the association between *DTNBP1* and methamphetamine psychosis in a case–control analyses.

Methods and Materials

Subjects

The subjects consisted of 197 patients (162 male, 35 female; mean age ± SD, 38.1 ± 12.6) with methamphetamine psychosis (MAP) and 243 age-, gender-, and geographic-origin-matched healthy control subjects (193 male, 50 female; mean age ± SD, 37.2 ± 12.0) who had no individual or family history of drug dependence or major psychotic disorders such as schizophrenia and bipolar disorders. All the subjects were unrelated Japanese who were born and lived in relatively restricted areas of Japan. All patients were outpatients or inpatients in psychiatric hospitals of the Japanese Genetics Initiative for Drug Abuse (JGIDA). Consensus diagnoses of methamphetamine psychosis were made by two trained psychiatrists according to the ICD-10 criteria on the basis of unstructured interviews and medical records. All healthy control subjects were also psychiatrically screened based on unstructured interviews. The study protocol and purpose were explained to all subjects participating in the study, and written informed consent was obtained from all subjects. This study was approved by the Ethics Committee of each participating institute of JGIDA.

The patients with methamphetamine psychosis were divided into subgroups according to three clinical phenotypes that may indirectly indicate the severity of and liability to psychosis:

1. *Latency to onset of psychotic state after initial methamphetamine consumption:* Median latency was 3 years; 99 (54.4%) of patients developed psychotic symptoms within 3 years of the first methamphetamine abuse, and 83 (45.6%) patients did after 3 or more years.
2. *Duration of the psychotic state after therapy:* Methamphetamine-induced psychosis (transient type) will usually subside within 10 days to 1 month following discontinuance of consumption and beginning of pharmacologic therapy with antipsychotics such as haloperidol or risperidone. Some patients show sustained (longer than 1 month) psychotic symptoms (prolonged type), however, regardless of detoxification from methamphetamine and adequate antipsychotic therapy (16,19). In our study, 107 (56.6%) patients showed the transient type of psychosis, and 82 (43.4%) patients showed the prolonged type of psychosis.
3. *Complication of spontaneous psychosis:* Once methamphetamine psychosis has developed, some remitted patients may experience spontaneous relapse due to nonspecific stresses, such as severe fatigue or life problems, without consumption of methamphetamine. The observation period for the presence or absence of spontaneous relapse was at least 1 year and averaged 12.3 ± 11.1 years. Eighty-three patients (42.8%) experienced spontaneous relapse, and 111 (57.2%) did not.

As to multisubstance abuse status, 37.2% patients concurrently abused other illicit drugs in addition to methamphetamine. Cannabinoids were most frequently abused (34.0%), followed by LSD (14.1%), cocaine (13.1%), opioids (12%), and hypnotics (9.9%). More than 60% of patients abused only methamphetamine, but about half had a past history of organic solvent abuse in their teenage years. All clinical data were obtained from interviews with patients and their families. Urine examination was not applied.

DNA Analysis

We genotyped the three single nucleotide polymorphisms (SNPs), P1655 (rs2619539), P1635 (rs3213207), and SNPA (rs2619538) of the *DTNBP1* gene that were examined previously by O'Donovan's group and were shown to have a significant association with both schizophrenia and psychotic bipolar disorders (12,13). They showed in the schizophrenia study that these three locus haplotypes showed the most significant results among 26 significantly associated haplotypes constructed by combinations of 9 SNPs of *DTNBP*. P1655 and P1635 were two of the markers that had provided the most significant results in the study by Straub *et al.* (1), and SNPA was reported to be significantly associated with schizophrenia in a Japanese population (9).

The genomic DNA was extracted from peripheral leukocytes using the phenolchloroform method. Genotyping was performed by the polymerase chain reaction (PCR)–restriction fragment length polymorphism method. Each polymorphic site was amplified by PCR in a volume of 15 mL containing 3% dimethyl sulfoxide and .75 units of Taq DNA polymerase (Promega, Japan) using a unique primer set (P1655 [mismatch]; 5′-ATCAGGCAAAATGATGTACTGC-3′, 5′-GCCTTTTAAATAATCCTATTAGCTATGAGAGT-3′, P1635; 5′-CTTTATGCAATAAGTATTCCTG-3′, 5′-GTATACCCTGTTTTAAGCAGAC-3′, SNPA; 5′-CCTGTTTCTCAACTTAGTACAC-3′, 5′-CCTTTATCTTATTTAACTCCTG-3′). PCR reaction was performed under the following conditions: 95°C for 5 min, then 35 denaturing cycles of 30 sec each at 95°C, 1 min of annealing at the appropriate temperature, and 30 sec of extension, and final elongation at 72°C for 10 min. The PCR products were digested with the corresponding restriction enzyme for each polymorphism, *Hin*fI for P1655, *Bse*NI for P1635, and *Ca*aI for SNPA, and then electrophoresed on 3.0% agarose gels and stained with ethidium bromide. All genotyping was performed in a blinded fashion, with the control and case samples mixed randomly. Part of the genotyping of P1655, P1635, and SNPA was confirmed by direct sequencing and a TaqMan SNP genotyping assay (C_16036968_10), respectively.

Statistical Analysis

Statistical analysis of association was performed using SNPAlyze software (Dynacom, Mobara City, Chiba, Japan). Deviation from Hardy–Weinberg equilibrium and the case–control study were tested using the χ^2 test. Linkage disequilibrium (LD) was tested using the χ^2 test, and D' and r^2 values were made the index in the authorization of LD. Case–control haplotype analysis was performed by the permutation method, and permutation p values were calculated based on 100,000 replications.

GenBank/EMBL Accession Numbers

Genome; NC_000006.10, NT_007592.14, MIM; 607145.

Results

The genotype distribution and allele frequencies for each polymorphism of patients with methamphetamine psychosis and control subjects are shown in Table 1. The genotype distributions of patients and control subjects did not deviate from the Hardy–Weinberg equilibrium at any of the three SNPs. We found a significant difference between patients and control subjects in the frequencies of the genotype or allele at P1635 and SNPA of

Table 1. Genotype and Allele Distribution of Three Single Nucleotide Polymorphisms of the *DTNBP1* Gene in Control Subjects and Patients with Methamphetamine (MAP) Psychosis

			Genotype				Allele		
		N	C/C	C/G	G/G	*p*	C	G	*p*
P1655	rs2619539								
Control		240	118 (49.2)	107 (44.6)	15 (6.2)		343 (71.5)	137 (28.5)	
MAP Psychosis		190	78 (41.0)	94 (49.5)	18 (9.5)	.17	250 (65.8)	130 (34.2)	.076
P1635	rs3213207		A/A	A/G	G/G		A	G	
Control		243	239 (98.4)	4 (1.6)	0 (.0)		482 (99.2)	4 (.8)	
MAP Psychosis		197	175 (88.8)	22 (11.2)	0 (.0)	.000025	372 (94.4)	22 (5.6)	.000030
SNPA	rs2619538		A/A	A/T	T/T		A	T	
Control		232	225 (97.0)	7 (3.0)	0 (.0)		457 (98.5)	7 (1.5)	
MAP Psychosis		197	182 (92.4)	15 (7.6)	0 (.0)	.046	379 (96.2)	15 (3.8)	.049

Numbers in parentheses indicate percentages.

the *DTNBP1* gene (P1635: genotype, $\chi^2 = 17.74$, $df = 1$, $p = .000025$; allele $\chi^2 = 17.20$, $df = 1$, $p = .000030$; SNPA: genotype $\chi^2 = 4.63$, $df = 1$, $p = .046$; allele $\chi^2 = 4.51$, $df = 1$, $p = .049$). The minor alleles of P1635 and SNPA, G and T alleles, respectively, were in excess in methamphetamine psychosis when compared with control subjects. To avoid a type I error due to multiple comparison, the Bonferroni correction was applied to the results. The G allele of P1635 was still significantly more frequent in the methamphetamine psychosis patients than in control subjects, but SNPA was not significantly different after correction. P1655 did not show significant differences in distribution of allele and genotype between groups.

Comparison between subgroups of the patients according to clinical phenotypes showed a significant difference in allelic and genotypic distribution of P1635 between the two subgroups divided by duration of psychotic state after therapy, transient and prolonged types (Table 2). The frequency of the minor allele G of P1635 was only 0.8% in control subjects, whereas it was 3.3% in patients with transient psychosis and 8.5% in patients with prolonged psychosis ($p = .027$, compared with transient psychosis). After Bonferroni correction, this was not significant. The other clinical phenotypes, psychosis latency and spontaneous relapse, were not associated with any SNP examined.

Estimation of the pairwise LD between the three SNPs of the *DTNBP1* gene using the D′ and r^2 values as an index showed that P1655, P1635, and SNPA have strong LD (D′ ranging between 0.65 and 1.0) with each other (Table 3). We then analyzed the three-marker haplotypes (Table 4) and found significant differences in patients and control subjects at P1655-P1635-SNPA ($\chi^2 = 27.8$, $df = 6$, global permutation $p = .0005$).

Table 2. Association of the *DTNBP1* Gene with Subgroups of Patients Divided by Clinical Phenotypes

		Genotype				Allele		
	N	C/C	C/G	G/G	*p*	C	G	*p*
P1655								
Latency to Onset of Psychosis, <3Y	96	35 (36.5)	50 (52.1)	11 (11.4)		120 (62.5)	72 (37.5)	
Latency to Onset of Psychosis, ≥3Y	79	36 (45.6)	37 (46.8)	6 (7.6)	.41	109 (69.0)	49 (31.0)	.20
Transient MAP Psychosis	103	44 (42.7)	50 (48.6)	9 (8.7)		138 (67.0)	68 (33.0)	
Prolonged MAP Psychosis	79	29 (36.7)	42 (53.2)	8 (10.1)	.71	100 (63.3)	58 (36.7)	.46
Spontaneous Relapse; No	108	41 (38.0)	54 (50.0)	13 (12.0)		136 (63.0)	80 (37.0)	
Spontaneous Relapse; Yes	77	34 (44.1)	38 (49.4)	5 (6.5)	.40	106 (68.8)	48 (31.2)	.24
P1635		A/A	A/G	G/G		A	G	
Latency to Onset of Psychosis, <3Y	99	89 (89.9)	10 (10.1)	0 (.0)		188 (94.9)	10 (5.1)	
Latency to Onset of Psychosis, ≥3Y	81	71 (87.7)	10 (12.3)	0 (.0)	.63	152 (93.8)	10 (6.2)	.64
Transient MAP Psychosis	107	100 (93.5)	7 (6.5)	0 (.0)		207 (96.7)	7 (3.3)	
Prolonged MAP Psychosis	82	68 (82.9)	14 (17.1)	0 (.0)	.022	150 (91.5)	14 (8.5)	.027
Spontaneous Relapse; No	111	98 (88.3)	13 (11.7)	0 (.0)		209 (94.1)	13 (5.9)	
Spontaneous Relapse; Yes	82	73 (89.0)	9 (11.0)	0 (.0)	.87	155 (94.5)	9 (5.5)	.88
SNPA		A/A	A/T	T/T		A	T	
Latency to Onset of Psychosis, <3Y	99	91 (91.9)	8 (8.1)	0 (.0)		190 (96.0)	8 (4.0)	
Latency to Onset of Psychosis, ≥3Y	82	75 (91.5)	7 (8.5)	0 (.0)	.91	157 (95.7)	7 (4.3)	.910
Transient MAP Psychosis	108	102 (94.4)	6 (5.6)	0 (.0)		210 (97.2)	6 (2.8)	
Prolonged MAP Psychosis	82	73 (89.0)	9 (11.0)	0 (.0)	.170	155 (94.5)	9 (5.5)	.18
Spontaneous Relapse; No	110	104 (94.5)	6 (5.5)	0 (.0)		214 (97.3)	6 (2.7)	
Spontaneous Relapse; Yes	82	74 (90.2)	8 (9.8)	0 (.0)	.26	156 (95.1)	8 (4.9)	.27

Number in parentheses indicate percentages.

Table 3. Pairwise Linkage Disequilibrium Between Single Nucleotide Polymorphisms of the *DTNBP1* Gene

	P1655	P1635	SNPA
P1655		.9643	1.0000
P1635	.0128		.6519
SNPA	.0114	.3522	

Right upper and left lower diagonal showed D′ and r^2 values, respectively.

The estimated haplotype frequency of C-A-A of P1655-P1635-SNPA was significantly lower in patients with methamphetamine psychosis than in control subjects (p = .0013). Conversely, the C-G-T haplotype was significantly higher in patients than in control subjects (p = .0012). Permutation p values of these haplotypes remained significant even after Bonferroni correction. Odds ratios were .62 (95% confidence interval [CI] .51–.77) and 14.9 (95% CI 3.5–64.2), respectively, indicating that the C-A-A haplotype protected against development of methamphetamine psychosis. On the other hand, the C-G-T haplotype was a significant risk factor for development of methamphetamine psychosis.

Discussion

We found a significant association between the *DTNBP1* gene and methamphetamine psychosis in individual marker and haplotype-based case–control analyses. The G allele of P1635 was shown to be a risk factors for methamphetamine psychosis. Numakawa *et al.* (9) reported that the G allele of P1635 was a risk factor for schizophrenia in Japanese; other reports have shown that it was also overtransmitted in Irish (1) but not in German schizophrenia (2). We also found that the G allele of P1635 was in excess in a subgroup showing a prolonged psychotic state, indicating that the allele was a risk for a worse prognosis of psychosis or refractoriness to antipsychotic therapy in patients with methamphetamine psychosis. The T allele of SNPA also showed a nominally significant risk for methamphetamine psychosis. Although it did not remain significant after multiple comparison correction, one study of schizophrenia showed that it was a significant risk (9), whereas another did not (13). The most striking findings in our study were that analyses of a haploype constructed by P1655-P1635-SNPA of the *DTNBP1* gene revealed a strong association with methamphetamine psychosis (p = .0005). The C-A-A haplotype was significantly more common in control subjects than patients with methamphetamine psychosis (p = .0013), implying a substantial protective factor given the odds ratio of .62. The protective haplotype found in our study of methamphetamine psychosis was identical with that previously reported in studies of schizophrenia and psychotic bipolar disorders (12,13). This evidence may indicate that the C-A-A haplotype of *DTNBP1* reduces the liability of individuals who suffer from endogenous psychoses or substance abuse to complications of psychotic symptoms such as delusions and hallucinations. Another possibility should be also considered, however; the C-A-A haplotype may be associated with methamphetamine dependence but not methamphetamine psychosis because all the patients examined in our study suffered not only from methamphetamine psychosis but also dependence. Accordingly, these hypotheses should be examined in other psychotic disorders—for example, psychotic depression, organic psychoses, and cocaine paranoia—as well as in other dependence disorders. In contrast, the C-G-T haplotype was a significant risk for development of methamphetamine psychosis. The frequency of the C-G-T haplotype was small at about 3% in methamphetamine psychosis but almost absent in control subjects, resulting in a strong risk and an odds ratio of 14.9. This haplotype was absent in the UK/Irish studies. In these studies, the C-A-T haplotype was a risk for both schizophrenia and psychotic bipolar disorder; however, this haplotype was rare (<1%) in Japanese samples and was not a significant factor for methamphetamine psychosis. In addition, the UK/Irish studies showed the G-G-T haplotype was as rare as 3% in control subjects but completely absent in schizophrenia, indicating a potent protective factor against schizophrenia. Again, this haplotype was absent in our samples. Such inconsistencies between the present study and UK/Irish studies indicate that the influence of genetic variation of *DTNBP1* on susceptibility to psychiatric disorders differs among the three distinct disorders (i.e., methamphetamine psychosis, schizophrenia, and psychotic bipolar disorder), although the protective C-A-A haplotype was common to all of them. In addition, population differences in SNP frequencies may also affect results. For example, the minor allele frequency of SNPA was .02, which was consistent with another Japanese study (9), but UK/Irish samples showed a frequency of .45 (13). The P1655 frequency was .28 in our samples, which was similar to another Japanese sample (.31) but different from Caucasian samples (.47 in Straub's study [1] and .49 in Williams's study [13]).

The relationship between abnormal dysbindin function and methamphetamine psychosis is unclear. The *DTNBP1* gene encodes a 40-Kd coiled-coil-containing protein that binds to β-dystrobrevin to form dystrophin-associated protein complex (DPC), which is found in postsynaptic densities of the brain (20). *DTNBP1*, however, is particularly expressed in certain axon terminals, notably, mossy fiber synaptic terminals in the cerebellum and hippocampus independent of DPC (20). Talbot *et al.* (10) found that patients with schizophrenia displayed a presynaptic DTNBP1 reduction in the hippocampus, and an inversely correlated increase in vesicular glutamate transporter-1 occurred in the same schizophrenia cases, suggesting a relationship between glutamatergic neurotransmission and DTNBP1. Evidence in vitro showed that overexpression of DTNBP1-enhanced glutamine release accompanied by an increase of presynaptic machinery SNAP25 and synapsin 1 and a knockdown of DTNBP1 by siRNA-reduced glutamate release. Reduced expression of DTNBP1 in schizophrenic brains may result in hypofunction of the glutamatergic system in the brain, which has been promising hypothesis for the pathophysiology of schizophrenia (21,22). Based on the clinical similarity between methamphetamine psychosis and schizophrenia, it has been assumed that shared neural mechanisms, not only dopamine systems but also gluta-

Table 4. Haplotype Frequencies of the *DTNBP1* Gene of Control Subjects and Methamphetamine (MAP) Psychosis

Haplotype P1655-P1635-SNPA	Controls Frequency	MAP Psychosis Frequency	Permutation p
C-A-A	.7101	.6046	.0013
G-A-A	.2741	.3315	.076
C-G-T	.0022	.0318	.0012
C-G-A	.0023	.0178	.11
C-A-T	.0073	.0055	.83
G-G-A	0	.0089	.15
G-A-T	.0039	0	.18

Haplotype analysis was performed by the permutation method. The global permutation p value was .0005.

mate systems, may be involved in the two psychotic disorders. Many lines of evidence from experimental studies using behavioral sensitization by repeated psychostimulant treatment, which has been recognized as an animal model of methamphetamine psychosis (18), showed pivotal roles of N-methyl-D-aspartate (NMDA) receptors and glutamate systems in the development of behavioral sensitization. Thus repeated administration of amphetamine or cocaine produces behavioral sensitization with enhanced efflux of glutamate in the ventral tegmental area (VTA) and accumbens, which are key brain structures for sensitization phenomena (23,24). NMDA receptor antagonists, including the noncompetitive antagonist MK-801, prevent behavioral sensitization to amphetamines when administered systemically or microinjected into the VTA (25–28). In contrast, phencyclidine, another NMDA antagonist, exacerbates amphetamine-induced abnormal behaviors and a hyperdopaminergic state in the prefrontal cortex and striatum (29–31). Amphetamines can also directly inhibit the NMDA receptor complex (32). Although the roles of NMDA receptors and glutamatergic systems in animal models of methamphetamine psychosis seem to be complex, our findings may indicate that variants of *DTNBP1* affect susceptibility to methamphetamine psychosis by implication of glutamatergic neurotransmission. In addition, DTNBP1 was shown to enhance phosphorylation of AKT protein by PI3-kinase and protect against neuronal cell death. Impaired PI3-kinase-Akt signaling and a genetic association with the *AKT1* gene were found in schizophrenia (20,33,34). Previously, we also found a significant association of the *AKT1* haplotype with the same patients of methamphetamine psychosis (35). It is possible that DTNBP1 confers susceptibility to methamphetamine psychosis via the PI3-kinase-Akt signaling cascade. In vitro evidence of interaction between dysbindin and dopamine system was recently reported. Kumamoto *et al.* (36) found that mRNA of disbindin expressed in the mouse substantia nigra, that suppression of dysbindin expression in PC 12 cells resulted in an increase of dopamine release, and that overexpression of dysbindin produced a tendency to decrease dopamine release. This finding suggests that dysbindin dysfunction may induce susceptibility to methamphetamine psychosis through interaction with dopamine systems.

Alternatively, the effect of DTNBP1 on cognitive ability should be considered. In an analysis of the phenotype–haplotype relationship, Williams *et al.* (13) found that the C-A-A protective haplotype was significantly associated only with higher educational attainment. A longitudinal study of childhood and adolescent antecedents of drug and alcohol problems in adulthood showed that, for both males and females, educational attainment was directly associated with a reduced risk for substance use problems (37). In this respect, higher educational attainment due to carrying the C-A-A haplotype might be involved in a reduced risk for methamphetamine psychosis, and the phenotype of higher educational attainment might be a common protective factor in methamphetamine psychosis and schizophrenia. Further studies are required to confirm this possibility.

Although our results remained significant after Bonferroni correction, it is possible that this was a chance finding resulting from reduced power due to small sample size. Analysis showed, however, that our sample size for the three SNPs had powers of .9994, 1.0000, and .9594 to detect an effect size (w = .1892, .5388, and .1263, respectively), with a significance level of .05 to detect significant associations in allelic analysis between control subjects and subjects with methamphetamine psychosis. Our total sample size is therefore large enough statistically, and it is unlikely that our positive findings results from reduced power. When methamphetamine psychosis patients are divided into subgroups according to clinical phenotypes, however, the statistical power may be reduced. It is possible that a rare haplotype C-G-T as a risk for methamphetamine psychosis may result from a chance fluctuation. In addition, a false-positive association owing to population stratification could not be excluded in this study despite careful matching of control subjects and patients. Our findings should be confirmed in larger samples and in different populations.

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shuffling gait. Because these side effects were extremely disturbing, aripiprazole was ultimately discontinued and substituted with risperidone 2 mg daily. Persecutory delusions attenuated significantly within five days, and parkinsonism gradually resolved after discontinuing aripiprazole.

Aripiprazole acts as a partial agonist at the D_2 and 5-HT_{1A} receptors and is also an antagonist at the 5-HT_{2A} receptor. The pharmacodynamics of aripiprazole is thought to be effective and well tolerated for schizophrenic patients [4]. However, several cases of aripiprazole-associated EPS have been reported [2,5]. For example, Cohen et al. [1] reported on two cases of depressive patients presenting with marked EPS, associated with concomitant use of aripiprazole and antidepressants (sertraline and venlafaxine). It was postulated that serotonergic agents with modest dopaminergic activity (e.g., sertraline) or noradrenergic activity (e.g., venlafaxine) might have modified aripiprazole's neurochemical effects.

In the case of Mr. A—a new-onset, drug-naïve schizophrenic patient—aripiprazole was the only agent prescribed. This medication, although partially effective, exerted intolerable EPS. Although it was possible that our patient's EPS were related to his genetic propensity for parkinsonism, our report suggests that a systemic investigation into aripiprazole's pharmacodynamic and side-effect profile would be welcome. We also recommend slow titration to target doses among some high-risk patients (e.g., young males) to decrease the risk of EPS and akathisia with aripiprazole in clinical settings.

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Successful Treatment of Severe Antidepressant-Induced Nausea with a Combination of Milnacipran and Olanzapine

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Nausea is one of the most problematic side effects induced by newer antidepressants, including selective serotonin reuptake inhibitors (SSRIs) and serotonin noradrenaline reuptake inhibitors (SNRIs). We report on a patient with obsessive-compulsive disorder and comorbid major depressive disorder who experienced severe nausea induced by SSRIs and an SNRI. She was successfully treated with a combination of milnacipran and olanzapine without nausea.

A 44-year-old female patient presented with the behavior of compulsively videotaping of entire TV programs on every channel. She recognized that this behavior was absurd and unnecessary, but its cessation made her anxious and irritable. When she could not record the TV programs herself, she asked her relatives to videotape them. When her relatives were not available, she asked video-recording companies to videotape the programs. Her room was filled with videotapes. She consulted a psychiatrist and was administered paroxetine. She could not continue this therapy, because a single paroxetine dose of 10 mg induced severe nausea. She visited several psychiatrists and was administered paroxetine, fluvoxamine, or milnacipran, with sulpiride as an antiemetic. However, the concomitant use of sulpiride could not suppress the severe nausea induced by antidepressants; a single dose of the smallest tablet resulted in intolerable nausea. After a psychiatrist told her that there was nothing to do for an obsessive-compulsive patient extremely sensitive to antidepressant-induced nausea, depressive symptoms appeared and gradually increased in severity. Finally, she was only able to videotape TV programs and spent most of the day in bed because of lassitude and loss of motivation. She visited our hospital on the advice of her relatives. She was administered a very low dose of milnacipran, because the incidence of nausea with milnacipran has been reported to be lower than that of SSRIs [5]. She was advised to buy a pill cutter and to try 1/8 of a 15-mg tablet once daily. This single low dose of milnacipran did not induce nausea. The number of doses per day and the amount of the dosage were gradually increased. She tolerated a dosage of 3.75 mg four times daily, but further increases in the daily dosage induced nausea. Because SSRI-induced nausea has been reported to be mediated via 5-HT_3 receptors [2], the concomitant administration of a drug with a 5-HT_3-inhibiting effect seemed to be appropriate. Olanzapine, an antipsychotic drug with a 5-HT_3-inhibiting effect, has been reported to be effective for obsessive-compulsive disorder in combination with SSRIs [1]. Therefore, a single dosage of 2.5 mg was added to the milnacipran treatment. After the administration of olanzapine, the daily dosage of milnacipran

could be gradually increased. When the daily dosage of milnacipran reached 90 mg/day, her depressive symptoms began to ameliorate. It became possible for her to do household chores and go outside for shopping. At that time, however, her excessive videotaping remained unchanged. When the daily dosage of milnacipran reached 150 mg/day, her excessive videotaping began to ameliorate. It became possible for her to cease videotaping new serial dramas. Currently, she is taking milnacipran 200 mg/day and olanzapine 2.5 mg/day. No apparent side effects have emerged. Her consumption of videotapes has become less than 10% of that at the first visit to our hospital.

Individual differences in pharmacokinetics and pharmacodynamics, mediated mainly via genetic polymorphisms, can lead to individual differences in susceptibility to nausea and other side effects induced by antidepressants as well as the antidepressant effect itself [4]. This case suggests the way that patients who are extremely sensitive to antidepressant-induced nausea should be treated. First, the initiation of antidepressants at a very low dosage should be considered. Intolerance of a single dose of the smallest tablet does not necessarily mean complete intolerance of an antidepressant. Second, the concomitant use of a 5-HT_3-blocking agent should be considered. Dopamine antagonists, such as sulpiride and metoclopramide, seem to be frequently used as antiemetics with antidepressants in Japan. However, their antidopaminergic effect is indirect for antidepressant-induced nausea. If the use of olanzapine is undesirable, mianserin, a tetracyclic antidepressant may be useful as a 5-HT_3 inhibitor [3].

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Rebound of Weight Gain Following Topiramate Cessation

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Glutamatergic system plays an important role in compulsive and/or addictive behaviors [5], which are associated with environmental cues [1]. Topiramate notably blocks the AMPA/kainate subtype of glutamate receptors [4]. Diverse studies and case reports have recently raised the interest of topiramate in the treatment of addictive disorders [6], obesity, and weight gain associated with antipsychotic drugs [2]. Despite this promising treatment for obesity [3], duration of treatment and its modality cessation remain understudied till to date.

We report here a case of weight rebound following topiramate cessation. Mr. A, a 28-year-old, Caucasian, consulted for the treatment of obesity (BMI = 32). He reported having received previously multiple dietary treatments, which was followed by weight gain and one unsuccessful orlistat treatment. Assessment of eating behaviors revealed no binge eating. The subject has 2 daily meals with 4–6 snacks equivalent to over half of daily caloric intake. Snacks were related to specific cues (such as specific food stimuli: sweets or chocolates and "small" sandwiches and chips...). He received four sessions in order to explore specific cues and determine their role in his obesity. When topiramate was started at a dose of 25 mg/day, he was advised to eat as usual, however, to observe his snacking and reducing it to twice daily as one of the treatment goals. Topiramate was then increased weekly by 25 mg, and by 50 mg/week from week 4 onwards, until changes were observed in eating behavior (snacking reduction ≥ 50%) at a dosage of 125 mg/day. Mr. A reduced food consumption within week 5, leading to a reduction in "snacking" to twice daily. He felt less attracted by food cues finding it easier to resist the temptation to snack, which was maintained at 1 to 2 times/day representing less than 1/4 of total daily caloric intake. He lost 5 kg by week 9. He then remained with that stable weight for a month. Topiramate dosage was increased to 175 mg/day. He experienced distressing, word-finding difficulties as well as 'mental block' sensations without further weight loss which led him to stop the treatment abruptly. Consequently, the distressing side effects disappeared within two days.

After 6 weeks, he consulted again describing a rebound of snacking (6–8/day) within the first week of topiramate cessation associated with weight gain (8.5 kg) and highest peak in body weight history. He then refused topiramate, afraid of a subsequent weight rebound and distressing side effects at higher dosages. A Cognitive Behavior Therapy (CBT) was then started leading to a moderate improvement during the four following months (3–4 snacks daily, weight loss 3.5 kg).

One can hypothesize that the observed weight rebound phenomenon could be prevented by more prolonged treatment, progressive discontinuation or adjunctive CBT. Topiramate discontinuation strategies should be studied. Furthermore, the addition of CBT in deconditioning the cues brought forth by topiramate and their

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The G196A polymorphism of the brain-derived neurotrophic factor gene and the antidepressant effect of milnacipran and fluvoxamine

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Abstract

Prediction of the response to different classes of antidepressants has been an important matter of concern in the field of psychopharmacology. The purpose of the present study was to investigate whether the G196A polymorphism of the brain-derived neurotrophic factor (BDNF) gene is associated with the antidepressant effect of milnacipran, a serotonin norepinephrine reuptake inhibitor, and fluvoxamine, a selective serotonin reuptake inhibitor. The subjects of our previous study of milnacipran ($n = 80$) and fluvoxamine ($n = 54$) were included in the present study. Severity of depression was assessed with the Montgomery Åsberg depression rating scale (MADRS). Assessments were carried out at baseline and at 1, 2, 4 and 6 weeks of treatment. Polymerase chain reaction was used to determine allelic variants. In all subjects receiving milnacipran or fluvoxamine, the *G/A* genotype of the BDNF G196A polymorphism was associated with a significantly better therapeutic effect in the MADRS scores during this study. When milnacipran and fluvoxamine-treated subjects were analysed independently, the *G/A* genotype group showed greater reduction of MADRS scores than other genotype groups, irrespective of which antidepressant was administered. These results suggest that the BDNF G196A polymorphism in part determines the antidepressant effect of both milnacipran and fluvoxamine.

Keywords

antidepressant effect, genetic polymorphism, fluvoxamine, major depressive disorder, milnacipran

Introduction

Prediction of the response to different classes of antidepressants has been an important matter of concern in the field of psychopharmacology. A consistent relationship between the antidepressant effect and the plasma concentrations of selective serotonin (5-HT) reuptake inhibitors (SSRIs) has not been obtained (Burke and Preskorn, 1999), although early pharmacokinetic studies identified significant relationships between the antidepressant effect and plasma concentrations of several tricyclic antidepressants (Perry *et al.*, 1987). In terms of serotonin norepinephrine (NE) reuptake inhibitors (SNRIs), venlafaxine showed a positive association between antidepressant efficacy and plasma concentrations (Charlier *et al.*, 2002), while this relationship was not observed for milnacipran (Higuchi *et al.*, 2003).

Recent progress in pharmacogenetics has facilitated investigation of the relationship between genetic polymorphisms and the antidepressant response. Genetic polymorphisms of the 5-HT and NE transporter have been investigated intensively, because they are believed to be the primary target of SSRIs and SNRIs. As a result,

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several interesting findings have been reported (Malhotra *et al.*, 2004; Yoshida *et al.*, 2004), but there is no consistent evidence to predict the antidepressant response. Thus, further pharmacogenetic studies of antidepressants must be performed in order to predict the antidepressant response adequately.

Recently, it has been proposed that antidepressants eventually cause critical genes to be activated or inactivated, no matter how they act on receptors and enzymes (Stahl, 2000). One of the likeliest candidate genes is brain-derived neurotrophic factor (BDNF), which belongs to a family of neurotrophic factors including neurotrophin-3/4/5 and nerve growth factor and has an important role as a potent modulator of synaptic transmission and plasticity. Substantial evidence supports that BDNF is involved not only in cognitive processes, such as memory and learning, but also in the pathophysiology of mood disorders and in the mechanism of antidepressant action, as follows. Expression of BDNF mRNA is down-regulated by either acute or repeated stressful conditions of immobilization (Smith *et al.*, 1995). An antidepressant effect in both the learned helplessness and the forced swimming tests is observed as early as 3 days after a single infusion of BDNF into the hippocampus (Shirayama *et al.*, 2002). Chronic treatment with tranylcypromine, a monoamine oxidase inhibitor, caused a significant increase in BDNF mRNA in the rat hippocampus (Russo-Neustadt *et al.*, 1999), and chronic administration of amitriptyline, a tricyclic antidepressant, significantly increased BDNF protein levels in the rat hippocampus and prefrontal cortex (Okamoto *et al.*, 2003). Thus, the BDNF gene is a plausible candidate gene for mood disorders and pharmacogenetic studies of the antidepressant response.

The G196A polymorphism in exon IIIA is located within the propeptide region of the BDNF gene. Several association studies have examined the G196A polymorphism and vulnerability for bipolar or major depressive disorders (Hong *et al.*, 2003; Nakata *et al.*, 2003). These studies have found no major role for the polymorphism in the pathophysiology of mood disorders, although Egan *et al.* (2003) reported that it influences human memory and hippocampal function. So far only one pharmacogenetic study of antidepressants and the BDNF G196A polymorphism has been carried out (Tsai *et al.*, 2003); in this study, the response to treatment with fluoxetine was evaluated for only 4 weeks and the response rate was as low as 33.6%.

In the current 6-week study, we examined the effect of the BDNF G196A polymorphism on the antidepressant effect of milnacipran, an SNRI, and fluvoxamine, an SSRI. In addition, we investigated another polymorphism of C132T in the non-coding region of exon V of the BDNF gene, which was detected and named C270T by Kunugi *et al.* (2001). Plasma concentrations of milnacipran and fluvoxamine were investigated to evaluate patients' compliance and an influence on the antidepressant effect.

Materials and methods

Subjects

The subjects in our previous studies (Yoshida *et al.*, 2002; Yoshida *et al.*, 2004) were included in the present study. The subjects were Japanese patients who fulfilled DSM-IV criteria for a diagnosis of major depressive disorder and whose scores on the Montgomery Åsberg depression rating scale (MADRS) (Montgomery and Asberg, 1979) were 21 or higher. Patients with other axis I disorders (including dementia, substance abuse, dysthymia, panic disorder, obsessive–compulsive disorder and generalized anxiety disorder) and those with axis II disorders determined by clinical interview were excluded. Patients with a history of childhood disorders were also excluded, as were patients with severe non-psychiatric medical disorders. The patients were 20–69 years of age and had been free of psychotropic drugs at least 14 days before entry into the study. After complete description of the study to the subjects, written informed consent was obtained. This study was approved by the Ethical Committee of Akita University School of Medicine. The clinical characteristics of the patients are shown in Table 1. There was no significant difference between responders and non-responders in regard to sex, age, number of previous episodes and presence of melancholia. There was no significant difference in clinical characteristics when milnacipran and fluvoxamine-treated patients were analysed independently (data not shown). The number of previous depressive episodes was very low. Indeed, most of the patients (milnacipran: 64/80, fluvoxamine: 41/54) were in their first episode.

Milnacipran treatment

Milnacipran was administered twice daily (the same dose after dinner and at bedtime) for 6 weeks. The initial total daily dose was 50 mg/day, and after a week it was increased to 100 mg/day. Patients with insomnia were prescribed brotizolam, 0.25 or 0.5 mg, a benzodiazepine sedative hypnotic, at bedtime. No other

Table 1 Clinical characteristics of the patients in the milnacipran and fluvoxamine treatment (responders and non-responders)

	Responders ($n = 85$)	Nonresponders ($n = 49$)		p
Sex (male/female)	34/51	16/33	$\chi^2 = 0.72$	0.40[a]
Age (years) (± SD)	50.7 ± 12.4	52.2 ± 12.8	$t = -0.68$	0.50[b]
Number of previous episodes (± SD)	0.48 ± 1.7	0.33 ± 0.7	$t = 0.77$	0.44[b]
Melancholia (+/-)	21/64	15/34	$\chi^2 = 0.55$	0.46[a]

[a] Analysis performed with the use of the χ^2 test.

[b] Analysis performed with the use of the unpaired t test.